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T7 PROMOTER PRIMER

CTAATACGACTCACTATAGGG

CTAATACGACTCACTATAGGG

GATTATGCTGAGTGATATCCC

cK XbaI PRIMER

CTGCAGGTCGACTGTAGAGGATCTACTAGT

CATGCCTGCAGGTCGACTCTAGAGGATCTACTAGT

GTACGGACGTCCAGCTGAGATCTCCTAGATGATCA

MUTAGENIC SITE XbaI

MUTAGENIC SITE

BamHI

TTCTGTGCTCTATGGTACAGCAACCTCTGGGTATTCCGT

AAGACACGAGATACCATGTCGTTGGAGACCCATAAGCCA

CACGAGATACCATGACGTTGGAGACCCATA

S95C PRIMER

CGTCGTGACTGGGAAAACC

GCAGCACTGACCCTTTTGG

GCAGCACTGACCCTTTTGG

U-19 PRIMER

pT7VICHA255

1. PCR REACTION WITH T7 PRIMER AND S95C
2. PCR REACTION WITH KXbaI PRIMER AND S95C
3. MIX PRODUCTS FROM EACH AT 95°C/10 MIN COOL TO 55°C

BamHI

4. FILL IN WITH POLYMERASE AND NUCLEOTIDES

BamHI

XbaI

5. AMPLIFY WITH PCR

BamHI

AND

BamHI

XbaI

FIG. 1.

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FIG. 2B.

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CTA CAA CTG AAT AGT CTG AGG TCT GAG GAC ACG GCC TTT TAT TTC TGT GCA AGT CAT CGG
GAT GTT GAC TTA TCA GAC TCC AGA CTC CTG TGC CGG AAC ATA AAG ACA CGT TCA GTA GCC
Leu Gln Leu Asn Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr Phe Cys Ala Ser His Arg

CDR3

	AGA	CGT	CGG	TTT	TGC	TG
TTT	GTT	CAC	TGG	GGC	CAC	GGG
AAA	GTG	ACC	CCG	GTG	CAC	GAC
Phe	Val	His	Trp	Gly	His	Gly
			Thr	Leu	Val	Thr
			Val	CAG	CAG	TGA
			ACT	GTC	ACT	GTC
			TCT	GCA	GCC	AAA
			CGG	CGG	TTT	TGC
			Ala	Lys	Thr	Thr
			Pro	Pro	Pro	Pro

FR4

CHL

FIG. 2B.

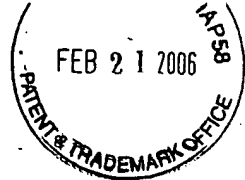


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SstI
CTCAGAGCTC
GCT GTT GTG ACT CAG GAA TCT
GCT GTT GTG ACT CAG GAA TCT
CGA CAA CAC TGA GTC CTT AGA
Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr Leu Thr
FR1
TGT CGC TCA AGT ATT GGG GCT GTT ACA ACT AGT AAC TAT GCC AAC TGG GTC CAA GAA AAA
ACA GCG AGT TCA TAA CCC CGA CAA TGT TGA TCA TTG ATA CGG TTG ACC CAG GTT CTT TTT
Cys Arg Ser Ser Ile Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys
CDR1
CCA GAT CAT TTA TTC ACT GGT GGT CTA ATA GGT GGT ACC AAT AAC CGG GCT CCG GGT GTT CCT
GGT CTA GTA AAT AAG TGA CCA CCA GAT TAT CCA CCA TGG TTA TTG GCC CGA GGC CCA CAA GGA
Pro Asp His Leu Phe Thr Gly Thr Gly Ile Ile Gly Thr Asn Asn Arg Ala Pro Gly Val Pro
CDR2
GCC AGA TTC TCA GGC TCC CTG ATT GGA GAC AAG GCT GCC CTC ACC ATC ACA GGG GCA CAG
CGG TCT AAG AGT CCG AGG GAC TAA CCT CTG TTC CGA CGG GAG TGG TAG TGT CCC CGT GTC
Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln
FR3

FIG. 3A.



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ACT GAA GAT GAG GCA AGA TAT TTC TGT GCT CTA TCG TAC TGC AAC CTC TGG GTG TTC GGT
TGA CTT CTA CTC CGT TCT ATA AAG ACA CGA GAT ACC ATG ACG TTG GAG ACC CAC AAG CCA
Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Cys Asn Leu Trp Val Phe Gly

CDR3

FR4

GGA GGA ACC AAA CTG ACT GTC CTA AGC CAG CCC AAG TCT TCG CCA TCA GTC ACC CTG TTT
CCT CCT TGG TTT GAC TGA CAG GAT TCG GTC GGG TTC AGA AGC GGT AGT CAG TGG GAC AAA
Gly Gly Thr Lys Leu Thr Val Leu Ser Gln Pro Lys Ser Ser Pro Ser Val Thr Leu Phe
TTT GAC TGA CAG GAT TCG

BsiWI

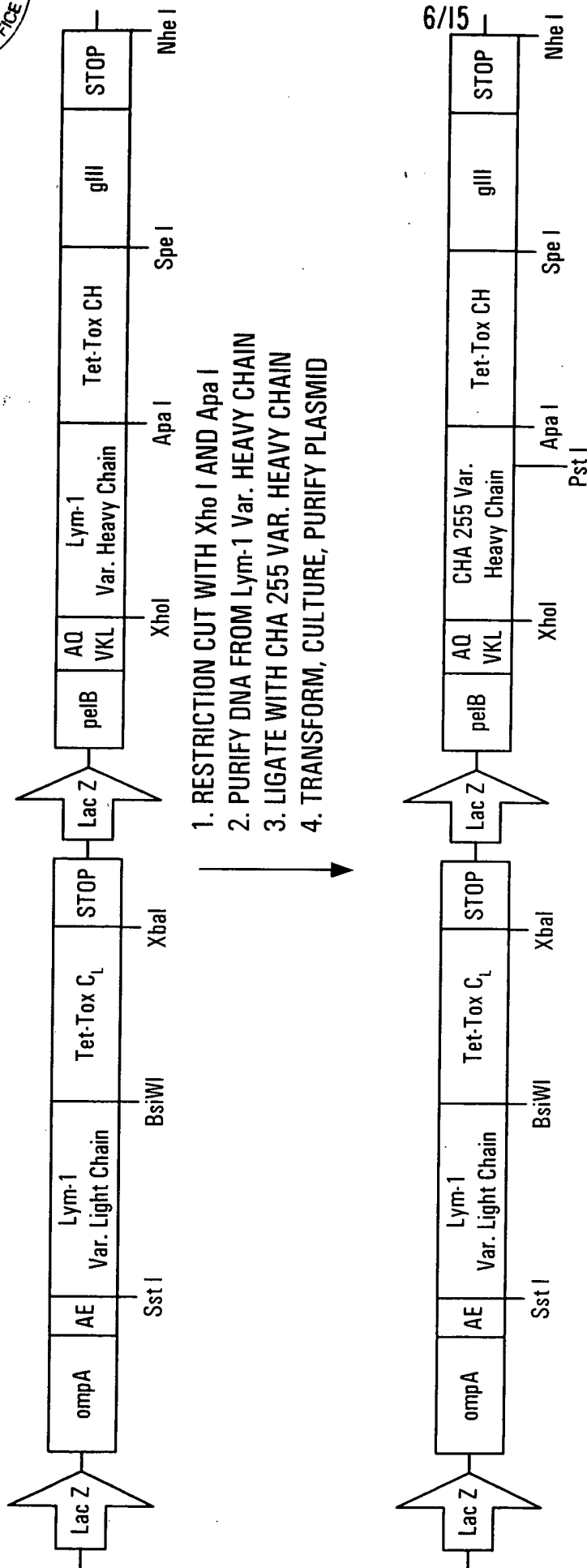
CGTACGCTC

CCG CCC TCC TCT GAA GAG CTA AGC TTG GGA ATC GGA TTC CCG GG
GGC GGG AGG AGA CTT CTC GAT TCG AAC CCT TAG CCT AAG GGC CC
Pro Pro Ser Ser Glu Glu Leu Ser Leu Gly Ile Gly Phe Pro Gly

FR4

CH1

FIG. 3B.

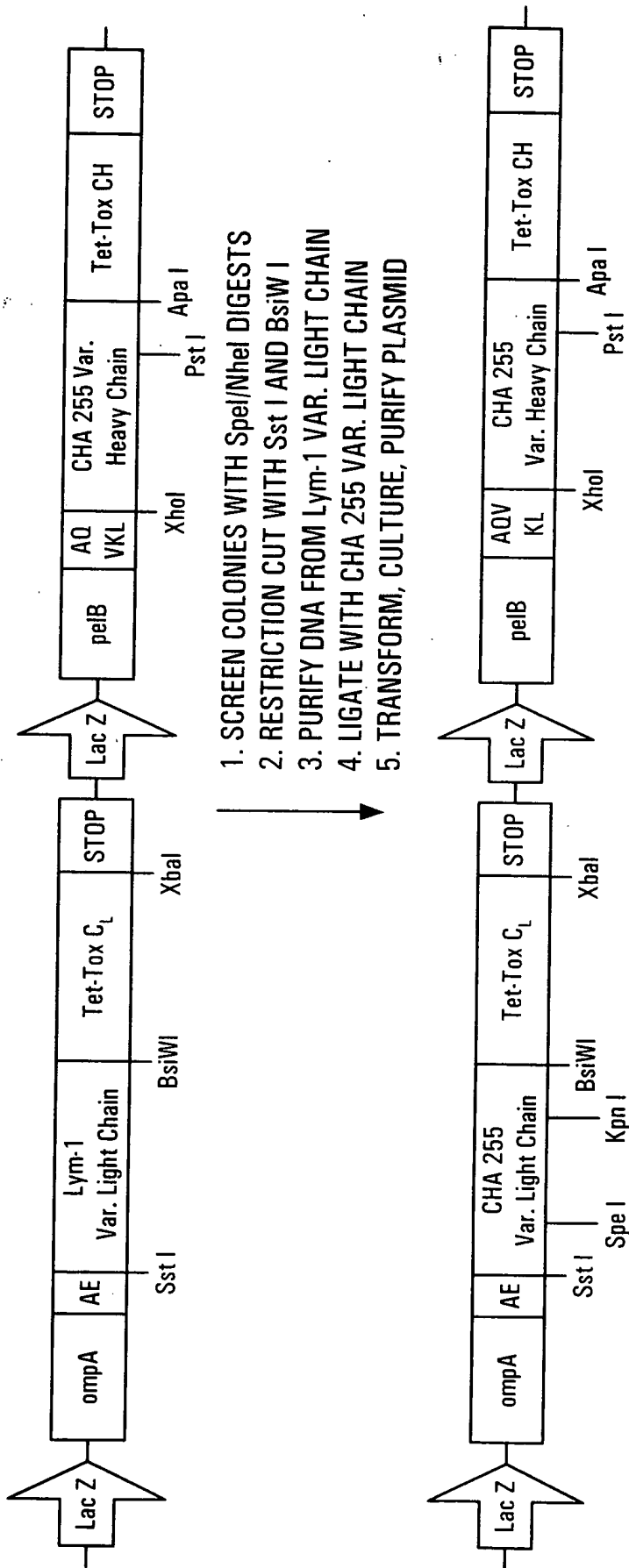


1. RESTRICTION CUT WITH Xho I AND Apa I
2. PURIFY DNA FROM Lym-1 Var. HEAVY CHAIN
3. LIGATE WITH CHA 255 VAR. HEAVY CHAIN
4. TRANSFORM, CULTURE, PURIFY PLASMID

1. SCREEN COLONIES WITH XhoI/PstI DIGESTS
2. SEQUENCE WITH HCF/GX PRIMERS
3. DIGEST POSITIVE COLONY WITH SpeI/NheI
4. LIGATE, TRANSFORM, CULTURE, PURIFY PLASMID

FIG. 4A.

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1. SCREEN COLONIES WITH SpeI/NheI DIGESTS
2. RESTRICTION CUT WITH Sst I AND BsiWI
3. PURIFY DNA FROM Lym-1 VAR. LIGHT CHAIN
4. LIGATE WITH CHA 255 VAR. LIGHT CHAIN
5. TRANSFORM, CULTURE, PURIFY PLASMID

1. SCREEN COLONIES WITH KpnI/XbaI DIGESTS
2. SEQUENCE POSITIVE CLONES WITH Lcf/Kx AND Hcf/Gx
3. EXPRESS PROTEIN AND CHARACTERIZE

FIG. 4B.

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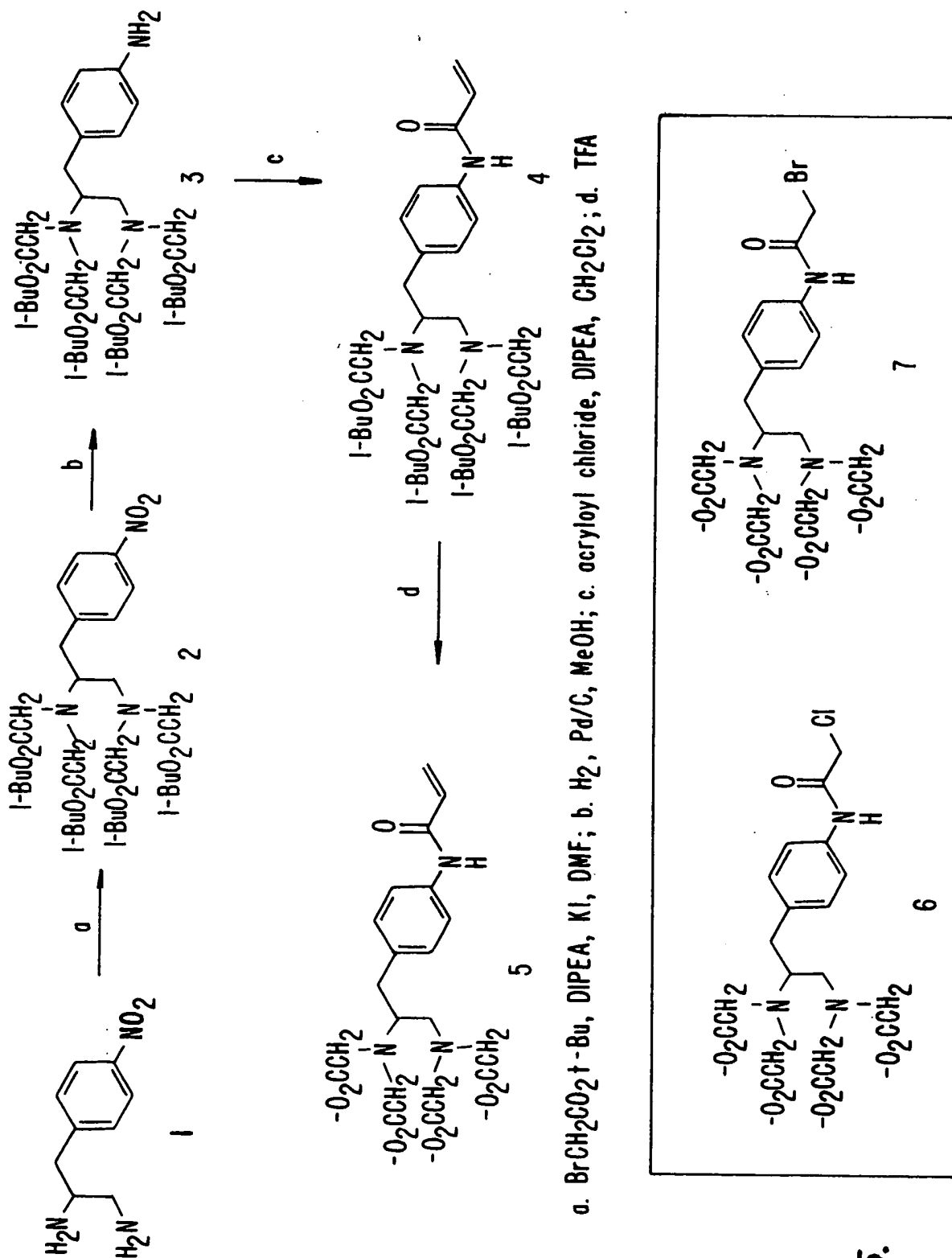
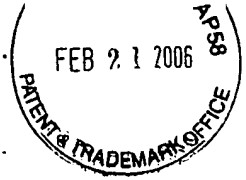


FIG. 5.



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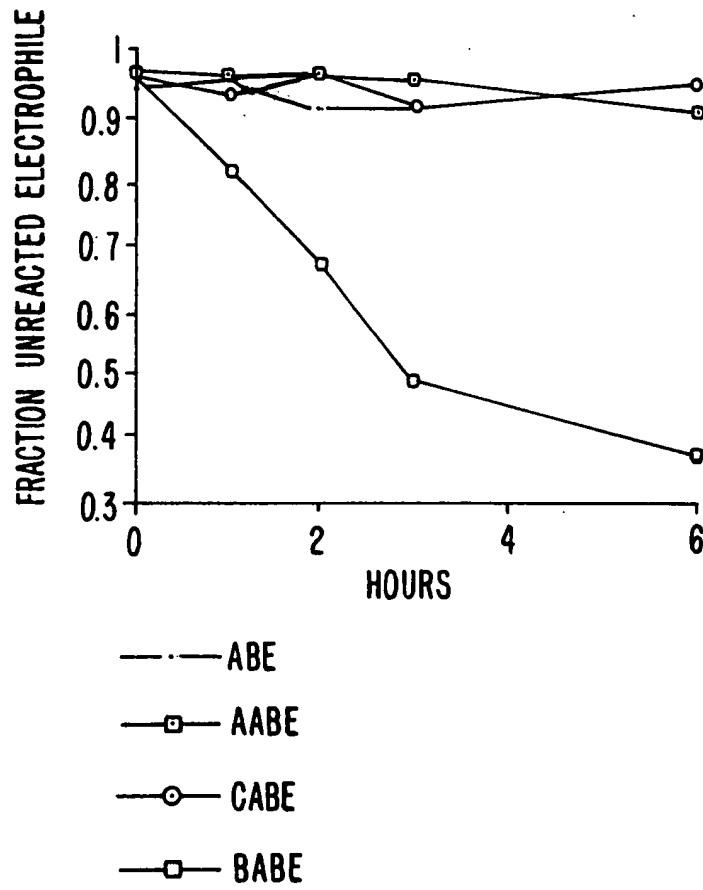


FIG. 6.

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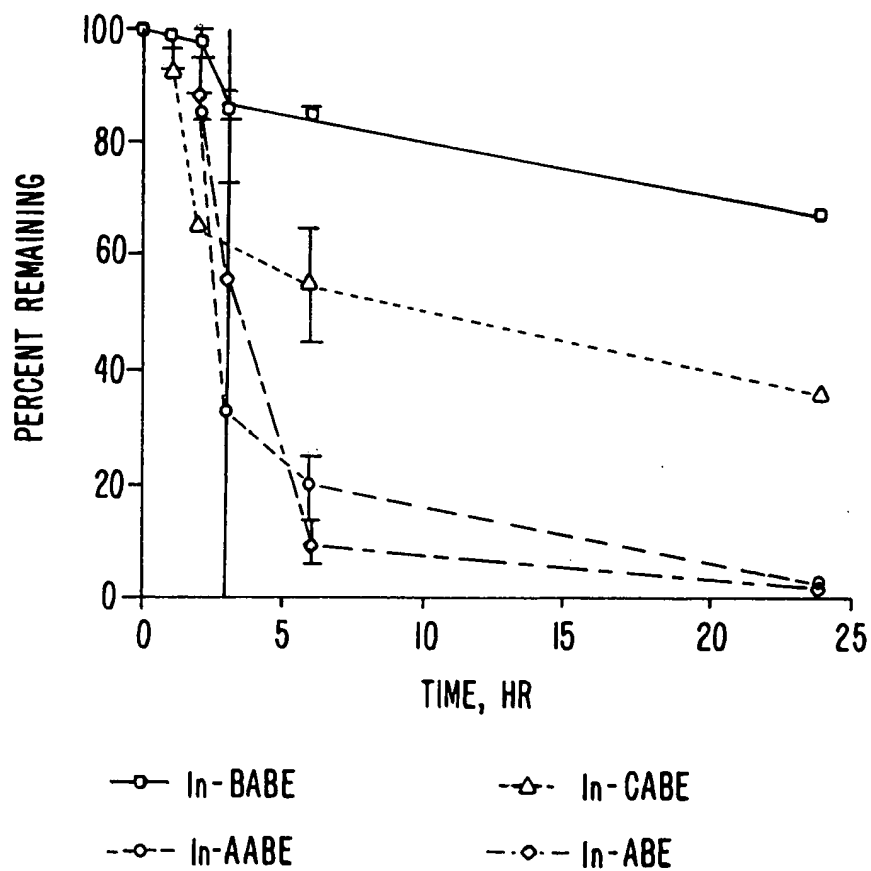
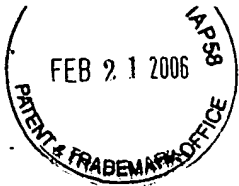


FIG. 7

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AGATCTGAAGTGACGCTGGTGGAGTCTGGGGGAGACTCAGTGAAGCCTGGAGGGTC
CCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTAAGTGGTGAAACCATGTCTTG
GGTTCGCCAGACTCCGGAGAAGAGGCTGGAGTGGGTGCAACCACTCTTAGTGGTG
GTGGTTTCACCTTCTATTTCAGCCAGTGTGAAGGGTCGTTTCACCATCTCCAGAGACA
ATGCCCAGAACAACCTCTATCTACAACCTGAATAGTCTGAGGTCTGAGGACACGGCCT
TGTATTTCTGTGCAAGTCATCGGTTTGTTCACTGGGGCCACGGGACTCTGGTCACTG
TCTCTGCAGCCAAAACGAOACCCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGA
GCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAAC
CGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCCG
CTGTCTACAGTCTCAAGACTCTACTTCCTCAGCAGCGTGGTGACCGTGCCCTTCA
ACAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC
AAGGTGGACAAGAAAGCAGAGCCCAAATCTTGTGACAAATCTAGAGGGCCCTTCGA
AGGTAAGCCTATCCCTAACCTCTCCTCGGTCTCGATTCTACGCGTACCGGTCATCA
TCACCATCACCATTGA

FIG. 8.

AGATCTGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGAAACAGTC
ACACTCACTTGTCGCTCAAGTATTGGGGCTGTTACAACTAGTAAGTATGCCAACTGG
GTCCAAGAAAAACCAGATCATTTATTCACTGGTCTAATAGGTGGTACCAATAACCGG
GCTCCGGGTGTTCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTC
ACCATCACAGGGGCACAGACTGAAGATGAGGCAAGATATTTCTGTGCTCTATGGTA
CTCCTGCCTCTGGGTRTTCGGTGGAGGAACCAAAGTACTGTCCTAAGCCGWACKGT
GGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC
TGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG
GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGG
ACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGAC
TACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGYTYGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

FIG. 9.

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AGATCTGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGAAACAGTC
ACACTCACTTGTCGCTCAAGTATTGGGGCTGTTACAACTAGTAACTATGCCAACTGG
GTCCAAGAAAAACCAGATCATTTATTCAGTGGTCTAATAGGTGGTACCAATAACCGG
GCTCCGGGTGTTCTGCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTC
ACCATCACAGGGGACAGACTGAAGATGAGGCAAGATATTTCTGTGCTCTATGGTA
CTCCAACCTCTGGGTGTTCTGGTGGAGGAACCAAACCTGACTGTCCTAAGCCAGCCCA
AGTCTTCGCCATCAGTCACCCTGTTTCCGCCCTCCTCTGAAGAGCTAAGCTTGGGAA
TCGGATTGCCGGGGTGCCTGCTGAATAACTTCTATCCCAGAGAGGGCCAAAGTACAGT
GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAG
GACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGA
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CCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

FIG. 10.

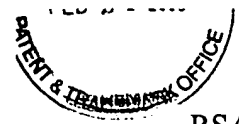
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ACACTCACTTGTCGCTCAAGTATTGGGGCTGTTACAACTAGTAACTATGCCAACTGG
GTCCAAGAAAAACCAGATCATTTATTCAGTGGTCTAATAGGTGGTACCAATAACCGG
GCTCCGGGTGTTCTGCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTC
ACCATCACAGGGGACAGACTGAAGATGAGGCAAGATATTTCTGTGCTCTATGGTA
CTCCAACCTCTGGGTGTTCTGGTGGAGGAACCAAACCTGACTGTCCTAAGCCAGCCCA
AGTCTTCGCCATCAGTCACCCTGTTTCCGCCCTCCTCTGAAGAGCTAAGCTTGGGAA
TCGGATTCCCAGGGGTGCCTGCTGAATAACTTCTATCCCAGAGAGGGCCAAAGTACAGT
GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAG
GACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGYTYGC
CCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

FIG. 11.

RSVVVTQESALTTSPGETVTLTCRSSIGAVTTSNYANWVQEKPDHLFTGLIGGTNNR
APGVPARFSGSLIGDKAALTITGAQTEDEARYFCALWYSLWVFGGGTKLTVLSRTV
AAPSVMFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSLXPVTKSFNRGEC

FIG. 12.

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RSVVVTQESALTTSPGETVTLTCRSSIGAVTTSNYANWVQEKPDHLFTGLIGGTNNR
APGVPARFSGSLIGDKAALTITGAQTEDEARYFCALWYSNLWVFGGGTKLTVLSRTV
AAPSVMFPPSDEQLKSGTASVVCLLNNFYPPREKQVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSXPVTKSFNRGEC

FIG. 13.

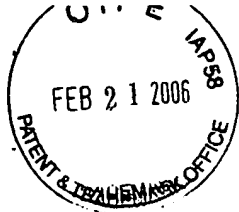
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APGVPARFSGSLIGDKAALTITGAQTEDEARYFCALWYCNLWVFGGGTKLTVLSRTV
AAPSVMFPPSDEQLKSGTASVVCLLNNFYPPREKQVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSXPVTKSFNRGEC

FIG. 14.

RSEVTLVEGRGDSVKPGGSLKLSCAASGFTLSGETMSWVRQTPEKRLEWVATTLSGG
GFTFYASAVKGRFTISRDN
AQNNLYLQLNSLRSEDALYFCASHRFVHWGHGTLTVSAAKTPPSVFPLAPSSKS
TSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSRLYFLSSVTVPFNSLGTQTYICNVNHKPSNTK
VDKKAEPKSCDKSRGPFEG
KPIPNLLGLDSTRTGHHHHHH

FIG. 15.

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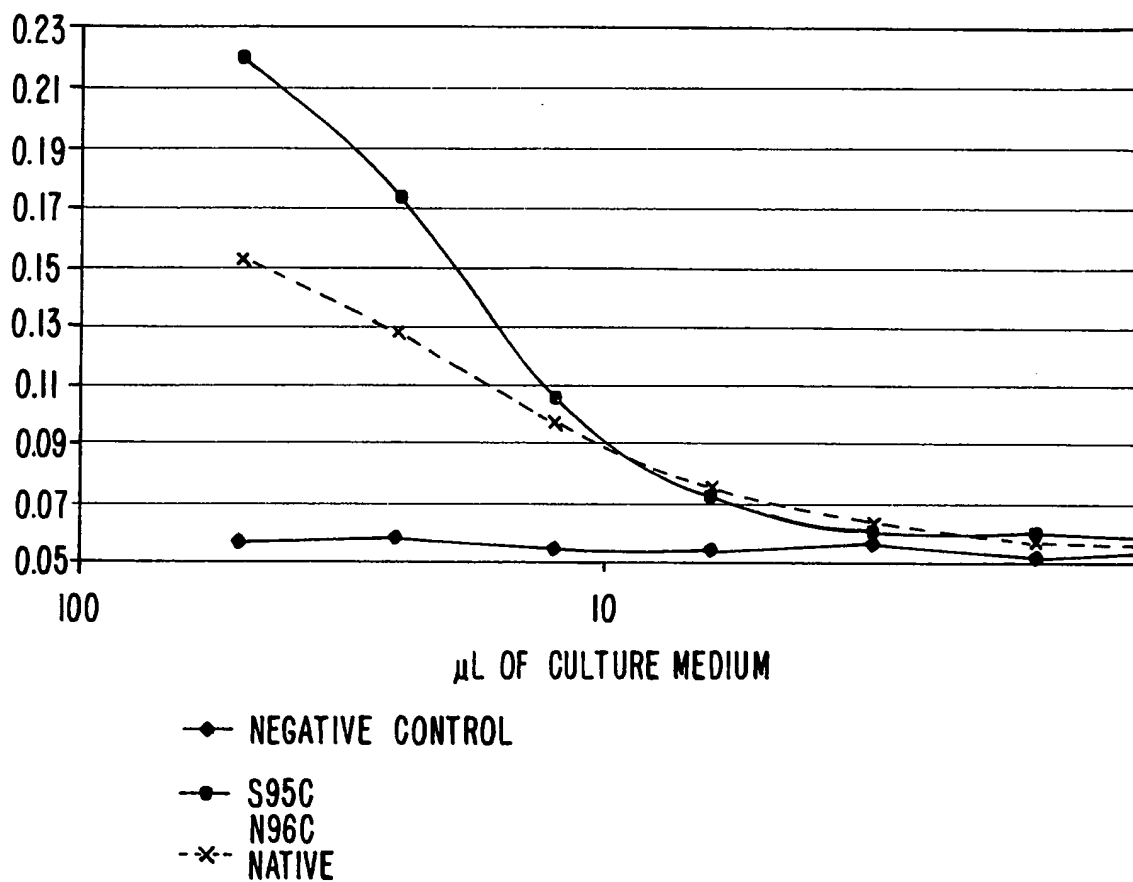


FIG. 16.

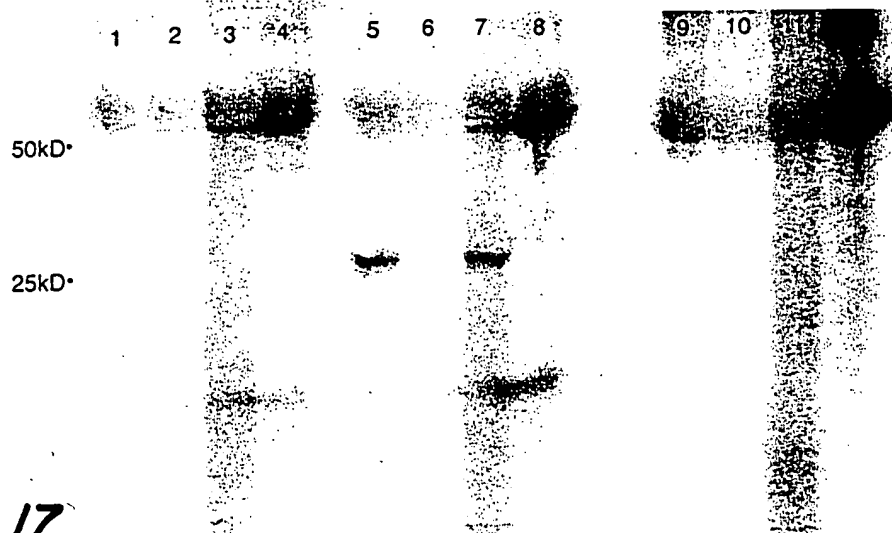


FIG. 17.

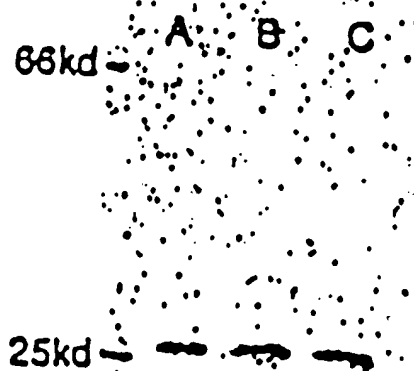


FIG. 18.

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